~~restriction site for the insertion of a heterologous target DNA sequence,~~ a transcription termination region functional in said plastid, and a 3' end of a plastid DNA sequence inclusive of the spacer sequence.

2. (Amended) The vector of claim 1 wherein ~~a~~ said vector further comprises at least one heterologous DNA sequence coding for ~~a~~ at least one molecule of interest ~~is inserted in one of the restriction sites~~.

3. The vector of claim 1 wherein said vector further comprises a ribosome binding site and a 5' untranslated region (5' UTR).

4. A vector of claim 1, wherein the antibiotic-free phytotoxic agent is a phytotoxic aldehyde and the detoxifying enzyme or protein is a aldehyde dehydrogenase capable of detoxifying said phytotoxic aldehyde.

5. (Amended) The chloroplast vector of claim 2 wherein ~~the~~ said at least molecule of interest is a polypeptide.

6. (Amended) The chloroplast vector of claim 3, wherein said plastid is ~~tobacco~~ a plant chloroplast.

7. A chloroplast vector which is described in Figure 1.

8. (Twice Amended) A vector of claim 4 for stably transforming the ~~cholorplast~~chloroplast genome where growth is inhibited by a phytotoxic aldehyde which is selected from the group consisting of acetaldehyde, formaldehyde, proprionaldehyde, butyraladehyde and betaine aldehyde.

9. (Amended) An integration and expression plastid vector for stably transforming a plastid genome of higher plant species where plant growth is inhibited by betaine aldehyde wherein

said integration and expression plastid vector comprises an expression cassette which comprises as operably joined components, a 5' ~~t~~end of a plastid DNA sequence inclusive of a 16S-23S spacer sequence, a promoter operative in said plastid genome, a DNA sequence encoding betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said betaine aldehyde in said cells to the corresponding non-toxic compound, a heterologous DNA sequence which codes for a molecule of interest, a transcription termination region functional in said plastid genome, and a 3' end of a plastid DNA sequence inclusive of the spacer sequence.

B4 cont.

10. (Twice Amended) A stably transformed plant which comprises a chloroplast which has been stably transformed with the vector ~~or the progeny of the vector~~ of Claim 9.

11. The stably transformed plant of claim 10, wherein the plant is a solanaceous plant edible for a mammal.

14. The stably transformed plant of claim 10, wherein the plant is a monocotyledonous plant~~,~~ selected from the group consisting of rice, wheat, grass, rye, barley, oat and maize.

15. The stably transformed plant of claim 10, wherein the plant is a dicotyledonous plant~~,~~ selected from the group consisting of soybean, peanut, grape, sweet potato, pea, canola, tobacco, tomato and cotton.

16. The stably transformed plant of claim 10, wherein the plant is a tobacco, tomato, potato, rice, brassica, cotton, maize or soybean plant.

17. The stably transformed plant of claim 10, wherein the plant is a homoplasmic plant.

18. (Twice Amended) The vector of any one of claims 2-3, 5, 7 or 9, wherein the selectable marker is driven by a promoter in green and non-green tissues selected from the group consisting of the 16SrRNA promoter, the psbA promoter, the ~~alpB~~atpB promoter, or the accD

B5

promoter.

B5 cont

19. (Amended) A method for introducing into plastid genome of a plant cell a DNA sequence encoding for detoxifying enzyme, which method does not require selection for successful transformants by the detection of antibiotic resistance, said method comprising introducing into cells of a plant species whose growth is inhibited by an antibiotic-free phytotoxic agent, an expression cassette which comprises as operably linked components, a 5' end of a plastid DNA sequence inclusive of a spacer sequence, ~~a promoter operative in said plastid,~~ a DNA sequence encoding a detoxifying enzyme acting as a selectable marker for transgenic plant cells and capable of detoxifying said phytotoxic agent in the cells to ~~the~~ a corresponding nontoxic compound, ~~a heterologous target DNA sequence,~~ a transcription termination region functional in said plant chloroplast cells, and the 3' end of the plastid DNA sequence inclusive of a spacer sequence.

20. (Amended) The method of claim 19 ~~wherein the~~further comprising introducing into the plastid genome of said plant cell at least one heterologous target DNA sequence ~~codes~~ coding for a molecule of interest.

21. (Amended) The method of claim 19 wherein the plastid DNA sequence codes for a phytotoxic aldehyde and the detoxifying enzyme or protein is an aldehyde dehydrogenase capable of detoxifying said phytotoxic aldehyde.

22. The method of claim 19 wherein the phytotoxic aldehyde is selected from the group consisting of acetaldehyde, formaldehyde, proprionaldehyde, butyraldehyde and betaine aldehyde.

23. A method of claim 19, wherein said method further comprises culturing said plant in a plant growth medium comprising said phytotoxic aldehyde, and selecting a plant cell that has had the DNA encoding sequence for a detoxifying enzyme introduced and hence is capable of growth

in the presence of said phytotoxic aldehyde.

24. The method of claim 23, wherein said method further comprises regenerating a transformed plant from said transformed plant cells.

25. The method of claim 21 wherein said phytotoxic aldehyde and the aldehyde dehydrogenase is ,betaine aldehyde dehydrogenase (BADH).

26. The method of claim 25, wherein said DNA sequence encoding a detoxifying enzyme is from sugar beet, or spinach plants.

27. The method of claim 25, wherein said DNA sequence is from a microorganism, E.coli.

B7

28. (Twice Amended) The method of claim 19, wherein the promoter is selected from the group consisting of 16SrRNA, psbA, accD and ~~alpB~~atpB.

30. The method of any one of claims 19-28, where the expression cassette further comprises a ribosome binding site (rbs) and a 5' untranslated region 5' UTR to enhance expression.

31. An integration and expression plastid vector competent for stably transforming the tobacco plastid genome where growth is inhibited by betaine aldehyde, a phytotoxic aldehyde, which comprises an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding spinach betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said phytotoxic aldehyde in the cells to glycine betaine, a heterologous DNA sequence which codes for a molecule of interest, a transcription termination region functional in said tobacco plastid, and a 3' part of a plastid DNA sequence inclusive of the spacer sequence.

Please add the following new Claim 32:

32. (New) The progeny of the stably transformed plant of Claim 10.

REMARKS

Applicant acknowledges the Examiner's rejection of Claims 1-12, 14-17, 19-28, and 30. Applicant further acknowledges the objection to Claims 13, 18 and 29. Applicant has canceled Claims 12-13 and 29, and added new Claims 31and 32 based on pages 10, and 12-13 of the Specification. No new matter has been added. Thus Claims 1, 11, and 14-30 are now pending in the application and Applicant asks for consideration of new Claims 31 and 32.

Applicant appreciates the Examiner's helpful suggestion in pointing out the numbering problem. Accordingly the Claims have been renumbered. The application, as filed, included an Abstract on a separate sheet as page 29. Further, the published PCT application included an abstract. Copies of both abstracts were enclosed with the Applicant's last reference. Applicant has also submitted in the previous Response, on a separate sheet, an abstract of the disclosure as required by 37 CFR 1.72(b) which is requested to be entered if the original cannot be found. Finally, the Applicant has corrected the priority in this case. The filing date of the provisional application was correct. However, the number of the application has been corrected to read "60/209,762" in the first paragraph of the Specification.

Turning now to the claim objections, Claim 13 and 29 have been canceled, and Claim 18 has been amended to eliminate incorrect multiple dependencies. Accordingly, Applicant requests that Claim 18 be treated on its merits. At the helpful suggestion of the Examiner the Applicant has added a proper article at the start of claims that remain under consideration, along with correcting other minor defects that were pointed out in the Examiner's objection.

Response to §112 Rejections

In response to the rejections based on 35 U.S.C. § 112, 1st paragraph, the Applicant

respectfully submits that as a result of the amendments to the claims, and also for the reasons set forth below in detail, this rejection is obviated.

The Applicant respectfully submits that the specification , as written, enables one skilled in the art to use Applicant's novel selection system in a variety of plants by interchanging a variety of aldehyde dehydrogenases capable of detoxifying a variety of corresponding phytotoxic aldehydes. The prevailing standard for determining whether the specification meets the enablement requirement is whether the experimentation needed to practice the invention is undue or unreasonable. *See* Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916). As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re* Wands, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988).

According to the aforementioned standard for determining enablement, Applicant respectfully submits that the details described in the specification provide enablement for plastid transformation vectors containing a gene coding for a variety of aldehyde dehydrogenases, as well as methods for transforming any of a number of plant species. Applicant notes for the Examiner's convenience that Ursin (U.S. Patent No. 5,633,153), which the Examiner has cited in this Office Action in a 103 rejection, was granted a claim to the genus covering a DNA sequence encoding an aldehyde dehydrogenase (Claim 1), by setting forth, as an example, the use of BADH (column 4, lines 34-40). Given this consideration and in further view of the remarks set forth, the Applicant asserts that the claims as amended are fully enabled.

The Applicant has incorporated by reference a universal vector capable of transforming the plastid genome of different plant species (page 9, lines 17-21). For the Examiner's convenience we

have included non-limiting extracts, which illustrate that the Applicant was in possession of a universal chloroplast vector.

The invention provides universal chloroplast integration and expression vectors which are competent to stably transform and integrate genes of interest into chloroplast genome of multiple species of plants. Transformed plants and their progeny are provided. Monocotyledonous and dicotylendonous plants are transformed which have never been transformed heretofore.

It has been discovered contrary to the conventional belief, that the chloroplast (ct) genome of plants contains spacer regions with highly conserved nucleotide sequences. The highly conserved nature of the nucleotide sequences of these spacer regions of the chloroplast genome makes such spacer regions, it has been discovered, ideal for the construction of vectors to transform chloroplasts of widely varying species of plant, this without the necessity of constructing individual vectors for different plants or individual crop species, which would require first a determination of the DNA sequence of each of the chloroplast genomes.

The Universal Vector. The invention has several useful embodiments. The invention provides a universal integration and expression vector hereinafter referred to as "UV" and its use for the expression of at least one phenotype in a variety of different plants.

The integration expression universal vector of the invention comprises an expression cassette (further described below) which comprises the necessary genetic elements to transiently or preferably stably transform the plastids e.g. chloroplast genome of a target plant cell with a foreign (heterologous) DNA coding sequence for a molecule of interest, like a phenotype to be expressed by the plant or a non-plant high value molecule, like a biologically active peptide (or polypeptide). The universal vector is constructed with a transcriptionally active region of a chloroplast genome that is highly conserved in a broad range of chloroplast genomes of higher plants. Preferably that region is the spacer 2 region; the intergenic spacer region between the t-RNA^{Ile} and the tRNA^{Ala} region. Such region is often referred to herein as a "spacer" region because in the chloroplast genome it is intergenic between several genes in the rRNA operon which is transcribed by one promoter. When built into the universal vector such region is generally referred to herein as a "border" or preferably as a "flanking sequence" or "flanking sequences". This is because in the universal vector, the operably joined genetic elements for transforming stably the plastid of the target plant are flanked on each side by a sequence i.e. a fragment of the spacer region. The flanking sequences in the vector and the spacer sequences in the chloroplast genome have sufficient homology to each other to undergo homologous recombination. The universal vector is inserted into the spacer of a transcriptionally active region in the

chloroplast genome. Generally, the spacer region is positioned in the inverted repeat region of the chloroplast genome. The rest of the construct, i.e. other than the flanking sequences and the expression cassette, is generally referred to herein as the "vector" which comprises bacterial sequences, like the plasmid cloning vectors pUC, pBR322, pGEM or pBlueScript.

At the time the application was filed, it was well-known to one skilled in the art that there were at least sixty transcriptionally-active spacer regions within the higher plant chloroplast genomes (Sugita, M., Sugiura. M., Regulation of Gene Expression in Chloroplasts of Higher Plants, Plant Mol. Biol., 32: 315-326, 1996). Specifically, Sugita et al. reported sixty transcriptionally-active spacer regions referred to as transcription units, as can be seen in Table II of the article. Applicant respectfully submits that because the transcriptionally active spacer regions were known, a universal vector could be used in the spacer regions contained within a variety of the higher plant chloroplast genomes. In light of Sugita et al., one skilled in the art knew where intergenic spacer regions were located in the plastid genome. Therefore, one skilled in the art could then use the method taught in the Applicant's specification to insert a universal vector within the spacer regions. In fact, the potato and tomato chloroplast genomes have been transformed using tobacco chloroplast DNA flanking sequences from the Applicant's universal vector (Nature Biotech, Vol. 19, 870-875 (2001), Ruf et al.; and Plant Journal, Vol. 19, 209-216 (1999), Sidirov et al.). Sidirov et al. and Ruf et al., support the Applicant's assertion that a universal vector can be used to insert genes into a variety of plants. Specifically, these references utilize the teachings of the Applicant to transform a plant other than tobacco. Therefore, transformation of multiple plant species is well known and understood by those skilled in the art. Heifetz et al. (2000, Biochimie 82: 655-666) teaches that complete plastid genomes have been sequenced from a variety of plant and algal species to provide a wealth of information regarding conservation of reading frames and regulatory sequences. Plastid-encoded

messages have also been found to be regulated post-transcriptionally over an unusually broad range. Taken together, these factors illustrate that heterologous genes or operons can be inserted into the plastid genome in a site-specific manner and can be expressed at levels ranging from low to extremely high, or alternatively, the coding sequences of endogenous plastid genes can be mutated in a directed fashion by virtue of the high efficiency of plastid homologous recombination. This is particularly true in light of the publication of the complete DNA sequence and genomic maps of at least fourteen different plant species. The following complete chloroplast genome sequences were already available in Genbank at the time of filing of this application:

Marchantia polymorpha 121,024 kbp

Nicotiana tabacum 155,844 kbp

Oryza sativa 134,525 kbp

Epifagus virginiana 70,028 kbp

Pinus thunbergii 119,707 kbp

Zea mays 140,387 kbp

Arbidopsis thaliana 154,478 kbp

Triticum aestivum 134,540

Euglena gracilis 143,172 kbp

Cyanophora paradoxa 135,599 kbp

Odontella sinensis 119,704 kbp

Porphyra purpurea 191,028 kbp

Chlorella vulgatis 150,613 kbp

Mesostigma viride 118,360 kbp

Thus one could, if so inclined, simply search appropriate spacer regions of the various plastid genomes without undue experimentation. A number of conserved intergenic spacer regions, among various plant species, are well known in the art. Sugita et al., "Regulation of Gene Expression in Chloroplasts of Higher Plants," *Plant Molecular Biology*, vol. 32, pp. 315-326, (1996). In addition, numerous incomplete chloroplast genome sequences are also available in the Genbank with more being added all the time.

Applicant kindly asks the Examiner to consider Example 18 from the "Revised Interim Written Description Guidelines Training Materials" (copy enclosed), where it is pointed out that "one skilled in the art would recognize that Applicant was in possession of all the various expression methods necessary to practice the claimed invention."

Example 18, a method for producing proteins, correlates with the Applicant's claims relating to selecting genetically engineered plants without the use of antibiotics as a selectable marker. In Example 18 the "*Neurospora crassa* mitochondria gene expression is essential to the function/operation of the claimed invention." Similarly, chloroplast expression of a selectable marker, which is a detoxifying enzyme, is essential to the function of the Applicant's claimed invention. Example 18 is drawn to an allowed genus despite the fact that there was only a single embodiment reduced to practice. In Example 18 there was only the expression of B-galactosidase. The mitochondria only expressed one protein with the help of one integration vector. Applicant's invention uses the universal vector PLD-BADH to transform tobacco plastid with BADH where the BADH functions as a selectable marker in the tobacco plastids. In other words, Applicant's embodiments are representative of a genus based on an antibiotic free selectable marker expression system.

Applying the reasoning of Example 18 to the Applicant's claims, any of a variety of detoxifying enzymes in the Specification, could be inserted into a tobacco plastid via the universal vector and then consequently tested for expression with a corresponding phytotoxic aldehyde. One skilled in the art would recognize that the Applicant was in possession of all the various expression methods necessary to practice the Applicant's invention.

Turning now to the assertion that Applicant has failed to disclose vectors besides the PLD-BADH vector, we ask the Examiner to consider page 9, lines 11-17 of Applicant's specification, Fig. 9A and 9B of the Applicant's specification, and the references relating as such, which were incorporated by reference. The aforementioned reference along with the specification teach a variety of transformation vectors capable of integrating detoxifying enzymes. More specifically, Figs. 9a and 9b provide examples of species specific vectors for alfalfa, soybean, potato and tomato which offer examples of plants whose DNA sequences were not known at the time of the filing of this application. Therefore, these examples teach one skilled in the art to create a species-specific vector without any detailed knowledge of DNA sequence of chloroplast genomes to be transformed.

Response to §112, 2nd Paragraph Rejections

Applicant has amended the claims to provide the proper antecedent basis, correct Markush format and omnibus claims, and clear up any indefiniteness of the claims. Further, Applicant has amended the method claims in order to avoid omitting essential method steps. As a result of these amendments, Applicant submits the claims are no longer indefinite and, hence, are now in a condition for allowance.

One of the objections to 1 and 19 pertains to the use of the phrase "protein acting as a

selectable marker," and how this would occur. As set forth in the specification, betaine aldehyde dehydrogenase is an enzyme which detoxifies betaine aldehyde. This can be used as a selectable marker by growing transformed plants and cells in the presence of an otherwise phytotoxic amount of betaine aldehyde. Transformed plants and cells survive due to the presence of the enzyme. Enzymes are proteins which catalyze chemical reactions, such as the detoxifying of betaine aldehyde. BADH is one such enzyme/protein.

Response to §103 Rejections

Applicant respectfully submits that solicited Claims 1-3, 5-7, 9-11, 14-21, and 23-30 are patentable over Maliga et al. (1999, U.S. Patent 5,877,402) in view of Rathinasabapathi et al. (1994, Planta 193:155-162). Applicant respectfully submits Maliga et al. teaches away from the use of genes encoding aldehyde dehydrogenases as selectable markers, as is suggested by Rathinasabapathi et al. Maliga et al. teach a *non-lethal* selectable marker gene which confers a selectable phenotype to cells containing plastids transformed with the DNA construct (Col. 4, lines 25-27). The Applicant has demonstrated that Maliga's "non-lethal selection" is not required for chloroplast transformation (page 6, lines 3-7).

Maliga et al. points out that selection markers have been identified by screening culture plant cells for *mutants* resistant to various antibiotics (Col. 3, lines 13-16). Furthermore, and more importantly, Maliga et al. teaches a chloroplast transformation system whose transformation process is performed using non-lethal selectable markers. Therefore, one skilled in the art would be led to believe that any derivative of Maliga et al's chloroplast transportation system would only function if using non-lethal selectable markers. Considering that it is well-

known in the art that accumulation of aldehydes are lethal to plant cells, one skilled in the art, upon reading Maliga, would believe non-lethal selection was required for chloroplast transformation, and thus aldehydes could not function as a non-lethal selectable marker.

We invite the Examiner's attention to all of Maliga et al.'s Examples 1-7, which all use a non-lethal selectable marker derived from *Nicotiana tabacum* and *Nitcotiana plumbaginifolia*. We further invite the Examiner's attention to Col. 15, line 64 through Col. 16, line15 wherein Maliga et al. defines a non-lethal marker.

> If the selectable marker is "non-lethal", transformants (i.e., cells expressing the selectable marker) will be identifiable by some means from non-transformants, but both transformants and non-transformants will live in the presence of the selection pressure.
>
> A selectable marker may be non-lethal at the cellular level, but at the organelle level; such is the case for the selectable marker preferred for use in the present invention. For example, the antibiotic spectinomycin inhibits translation of mRNA to protein in plastids, but not in the cytoplasm. Plastids sensitive to spectinomycin are incapable of producing proteins comprising the photosynthetic apparatus, as well as other proteins required for plastid survival. In the presence of spectinomycin, cells comprising such plastids are kept alive by growing them under photoheterotrophic conditions (i.e., supplying an exogenous carbon source), but the plastids therein do not survive, and the cells or tissues are bleached white, instead of being green.

Specifically, Maliga et al. refers to non-lethality at the cellular level, wherein sensitive cells or tissues can be grown in the selective medium, but resistant tissues are readily identifiable therefrom. In other words, both transformants and non-transformants **live** in the presence of selection pressure. In sharp contrast, the Applicant specifically demonstrates that under betaine aldehyde selection, only transformed cells live while untransformed cells die. Applicant confirms that betaine aldehyde is toxic to plant cells and, therefore, betaine aldehyde offers lethal selection.

Consequently, one skilled in the art, after reviewing the teachings of Maliga et al., would be led away from using BADH or another aldehyde dehydrogenase, as a selectable marker, because Maliga et al. teaches only the use of non-lethal selectable markers.

Furthermore, it was not obvious to transform plastids using BADH as a selectable marker with the transformation vectors taught by Maliga et al. While it was known that BADH was a plant enzyme, it could not be conclusively demonstrated BADH was a chloroplast-generated enzyme. BADH lacks the typical transit peptide found in all chloroplast proteins imported from the cytosol. The absence of these typical transit peptides would leave one skilled in the art questioning whether the proper cleavage of BADH enzyme in the stroma within plastids would leave the BADH fully functional. One skilled in the art could only speculate whether the BADH enzyme would be catalytically active, without the proper cleavage within plastids. Thus one skilled in the art could not introduce the gene for BADH into a plastid with the expectation that BADH would be produced, and function to detoxify betaine aldehyde. Logically, it follows that if the BADH is not catalytically active within the plastid, it could not serve as a selectable marker. We invite the Examiner's attention to *In Re Lalu*, 747 F.2d 703, 223 U.S.P.Q.1257 (Fed. Cir. 1984), which held that there is no motivation to substitute a claimed compound for a prior art compound, unless the two compounds have a *common utility*. *In Re Lalu* is analogous to the current situation because there is no suggestion that the selectable markers exemplified in Maliga et al. share any common utility, structure or origin with the BADH selectable marker.

At the very most, it was only obvious to try utilizing BADH as a selectable marker in the plastid transformation vectors disclosed in Maliga et al. Prior to the Applicant's invention no plastid genome had been modified without the use of antibiotic selection markers. Rathinasabapathi et al.

merely pointed out that the use of BADH as a selectable marker was of interest, and therefore Rathinasabapathi offers no suggestion of success that chloroplast could integrate BADH. Rathinasabapathi et al. observed that the BADH *enzyme*, not the gene, is targeted to the chloroplasts. Rathinasabapathi et al. does not teach that BADH can be stably integrated into the plastid genome to produce a functional enzyme. Applicant has discovered that the BADH gene can be integrated into the chloroplast gene.

> The strategy to distinguish between nuclear and chloroplast transgenic plants was to land one primer (3P) on the native chloroplast genome adjacent to the point of integration and the second primer (3M) on the aadA gene (Figure 1). This primer set generated 1.6 kb PCR product in chloroplast transformants (Figure 4). Because this product cannot be obtained in nuclear transgenic plants, the possibility of nuclear integration can be eliminated.

Without knowledge that the BADH gene was integrated into the chloroplast genome, rather than simply expressed in it, one skilled in the art would have no expectation that Maliga et al's chloroplast transformation vector and methods could successfully integrate BADH into the chloroplast genome of plants. The importance of chloroplast *gene* integration has been articulated in the specification.

> Plastid genetic engineering, particularly chloroplast genetic engineering, is emerging as an alternative new technology to overcome some of the environmental concerns of nuclear genetic engineering (reviewed by Bogorad, 2000). One common environmental concern is the escape of foreign gene through pollen or seed dispersal from transgenic crop plants to their weedy relatives creating super weeds or causing genetic pollution among other crops (Daniell 1999B). Keeler et al. (1996) have summarized valuable data on the weedy wild relatives of sixty important crop plants and potential hybridization between crops and wild relatives. Among sixty crops, only eleven do not have congeners and the rest of the crops have wild relatives somewhere in the world. In addition, genetic pollution among crops has resulted in several lawsuits and shrunk the European market of Canadian organic farmers (Hoyle 1999). Several major food corporations have required segregation of native crops from those "polluted" with transgenes.

In fact, Rathinasabapathi et al. performed their experiment using antibiotic (specifically kanamycin) resistance, as selection markers (Col. 1, page 157). Rathinasabapathi et al. used five BADH construction vectors, which exhibited significant variation in BADH expression among the constructs (left column, page 157). This illustrates that one skilled in the art would not find it obvious that BADH would be consistently expressed through the transformation vectors disclosed in Maliga et al.

Solicited Claims 1-3, 5-7, 9-11, 14-21 and 23-30 have been rejected under 35 U.S.C. §103 based on Maliga et al. in view of Ursin (1997, U.S. Patent 5,633,153). Applicant respectfully submits that the aforementioned claims as amended are patentable over Maliga et al. in view of Ursin.

Applicant respectfully submits that Ursin teaches a method of using an aldehyde dehydrogenase as a selectable maker for *nuclear* transgenic plants. Ursin discloses a DNA construct coding for an aldehyde dehydrogenase through eukaryotic promoters used for *nuclear* transformation and culturing such transformed cells in growth media containing the corresponding phytotoxic aldehyde; the transformed plants demonstrate resistance to the phytotoxic aldehyde. The Examiner's attention is invited to Col. 5, lines 18-40 of Ursin et al. where it is clear that Ursin et al. teaches away from chloroplast expression of BADH:

> Plant BADH genes are nuclear encoded, but the proteins are transported to the chloroplasts for accumulation of glycine betaine. The chloroplast targeting mechanism for these proteins has not been clearly established. Protein purification and sequence analysis indicates the presence of an 8 amino acid transit peptide, which is atypically short compared to other known transit peptides. For detoxification of betaine aldehyde provided in plant cell culture media, production of BADH in the cytoplasm of transgenic plant cells may provide for higher selection efficiency. The cytoplasm of cells in direct contact with media containing betaine aldehyde likely accumulate high levels of betaine aldehyde in the cytoplasm, and hence, metabolism of the betaine aldehyde into glycine

> betaine via the action of BADH, would effectively remove the betaine aldehyde from the cell. There are several approaches that could be used to provide for localization of the BADH protein in the cytoplasm. For example, a BADH gene from an organism which does not require plastid targeting mechanisms could be used, such as BADH from *E. coli* or yeast. Where plant BADH genes are used, a construct in which the BADH transit peptide region is remove(d) can be prepared. Alternatively, one could use a translational fusion construct between the BADH cDNA and a protein or peptide fragment that destroys chloroplast targeting and encodes a functional BADH protein.

Specifically, Ursin et al. suggests several approaches to expressed BADH enzyme outside of the chloroplasts. For example, Ursin et al. teaches use of BADH enzyme without a transit peptide from either yeast or *E.coli*, or deletion of the transit peptide, or use of a fusion protein which destroys chloroplast targeting. Furthermore, Ursin et al. suggests that production of BADH in a cytoplasm provides higher selection efficiency than expression within chloroplasts, and as a result teaches away from expression of BADH within chloroplasts. Finally, Ursin et al. also suggests that presence and use of an 8-amino acid transit peptide is not adequate for targeting BADH to the chloroplast. The Applicant has demonstrated that Ursin et al.'s suggestions are not true.

Applicant's invention, however, has shown not only where BADH is expressed, but also, that it is of great significance that BADH is expressed in plastids rather than the nucleus. Nuclear BADH, having a high GC content could not be expected to express well in AT-rich prokaryotic plastid compartments, because the codon usage is very different between the prokaryotic chloroplast compartment and the eukaryotic nuclear compartment. Therefore, it was not obvious to express a nuclear gene in a plastid compartment.

Applicant respectfully submits that solicited Claims 1-3, 5-7, 9-11, 14-21 and 23-30 are patentable over Maliga et al. in view of Holmstrom et al. (1994, Plant J. 6: 749-758).

Holmstrom et al. fails to teach a spinach or sugar beet BADH gene direct to plant chloroplast in the *absence* of a typical transit peptide. Examiner's attention is invited to the following passage of Holmstrom et al. (pg. 753 left column)

> The plants with BADH localized to chloroplast exhibited clearly reduced root growth while the transformants with the enzyme in the cytoplasm showed normal root growth. A possible explanation for this phenotype is that betaine aldehyde is not effectively transported tot he chloroplast where it could be detoxified.

As a result it is clear that Holmstrom et al. did not teach plants which were resistant to betaine aldehyde, although plants localized BADH in the cytoplasm rather than within the chloroplast as the Applicant has done.

As with Rathinasabapathi et al., Holmstrom et al. merely indicates that BetB protein was incorporated into and processed inside the chloroplast. Applicant, however, has discovered that BADH is actually transcribed and expressed in chloroplasts. As was explained in the specification, there are a number of benefits to having chloroplasts transformed with a DNA sequence as opposed to nuclear DNA transformants. The combination of Maliga et al. and Holmstrom et al. would cause one skilled in the art to be discouraged from attempting to use BADH genes of plant origin in chloroplasts via Maliga et al's expression vectors.

Holmstrom et al. states that "*E.coli* betaine biosynthetic enzymes appear to be more related to the corresponding mammalian enzymes than to plant ones" (Left Col. Page 750). This disclosure further discourages one skilled in the art from attempting to insert plant BADH genes into chloroplasts.

Holmstrom et al. illustrates that BADH directed into the chloroplast resulted into plants which were not resistant to betaine aldehyde because those plants had BADH enzyme which was

located in the cytoplasm. As a result, Holmstrom et al. was teaching against chloroplast expression of BADH.

In light of the foregoing, Applicant respectfully submits that Claims 1-3, 5-7, 9-11, 14-21 and 23-30 are patentable over Maliga et al. in view of Holmstrom et al. and further respectfully submits that the claims are now in complete condition for allowance, which is respectfully requested.

Respectfully submitted,

T. Daniel Christenbury
Reg. No. 31,750
Attorney for Applicant

TDC/JEB:gj
(215) 656-3300